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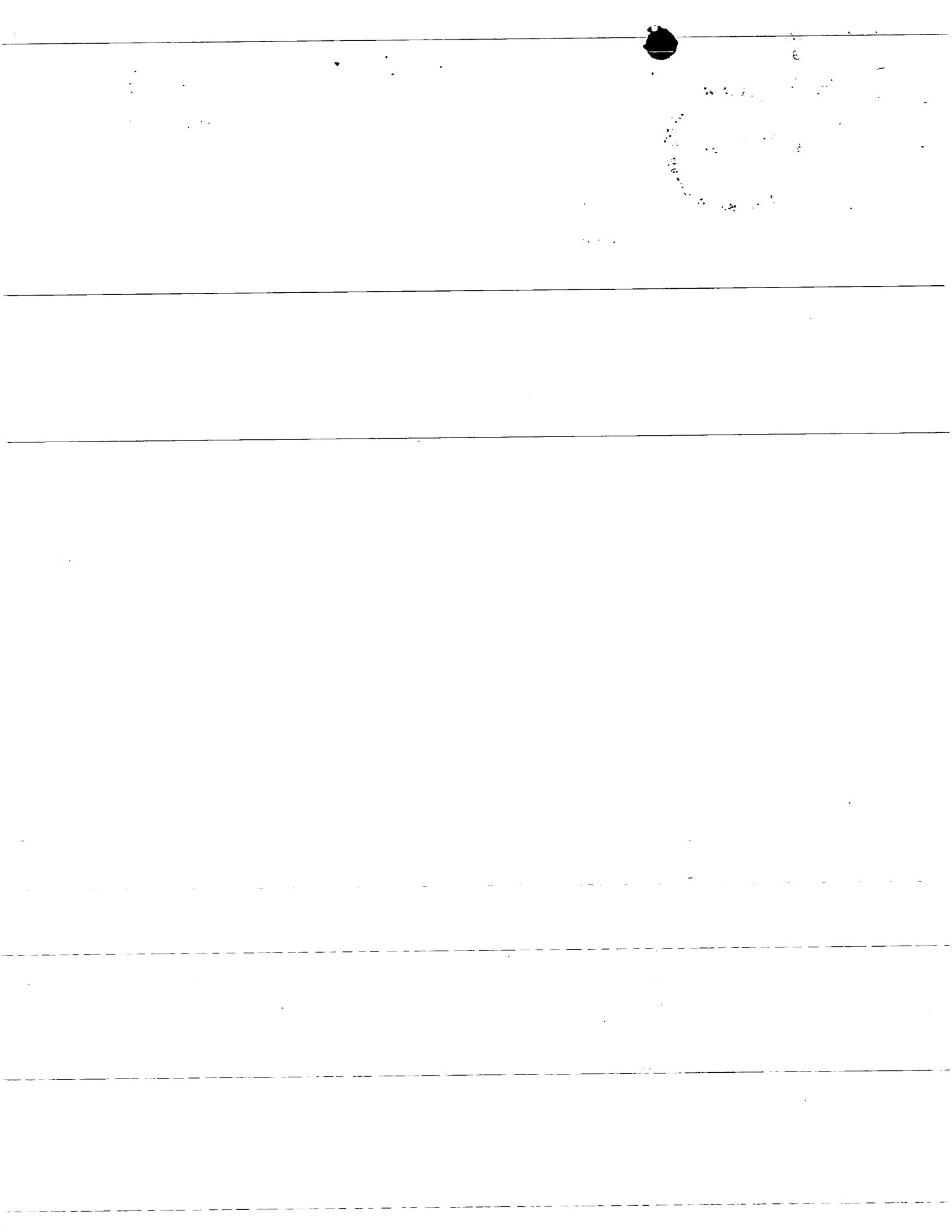

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University of Leicester
University Road
Leicester
LE1 7RH

Patents ADP number *(if you know it)* 798348 001

If the applicant is a corporate body, give the country/state of its incorporation — Great Britain

4. Title of the invention — Clq Receptor

5. Name of your agent *(if you have one)*

McNeight & Lawrence
Regent House
Heaton Lane
Stockport
Cheshire SK4 1BS

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Patents ADP number *(if you know it)* 00011150011

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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C1q Receptor

The present invention concerns novel uses of the C1q Receptor (C1qR) binding domain and inhibitors thereof.

The C1qR binding domain within C1qR has previously been identified (Stuart, G.R. *et al.*, 1996, FEBS Letters, 397: 245-249 and references therein). The C1qR homologue Calreticulin (CaR/CRT) has also been identified and shown to have very high sequence homology, and as such reference to C1qR is considered to also be reference to CRT and *vice versa* unless otherwise stated.

Certain functionality has been attributed to C1qR upon its activation by the binding of C1q, namely immunological responses such as phagocytosis, enhanced cytokine and antibody production and antibody-dependent cell cytotoxicity. C1qR is also known to bind the collectin proteins SP-A, MBL, CL43 and conglutinin. However, the exact nature of C1qR has not yet been determined nor its structure identified. Sequence analysis does not identify it as being part of a known class of cell-surface receptors.

The present inventors have now found that the C1qR binding domain is in fact a CUB (Complement Ubiquitin) domain, and as such certain previously unknown functionality can be attributed to C1qR and inhibitors of same. CUB domains are well known (see for example Day, A.J. *et al.*, 1993, Behring Inst. Mitt., 93: 31-40; Thiel, S. *et al.*, 1997, Nature, 386: 506-510; Arlaud, G.J. *et al.*, 1993, Behring Inst. Mitt., 93: 189-195).

Thus according to the present invention there is provided the use of a C1qR binding domain as a CUB d main.

Sequence analysis of C1qR shows little primary homology with known CUB domains, but the C1qR binding domain does in fact have 6 to 7 consensus residues (out of a total of about 100) with CUB and this provides the C1qR binding domain (and thus C1qR) with CUB domain functionality.

The C1qR binding domain may form part of an existing molecule, for example C1qR or it may form part or the whole of a novel molecule, for example a molecule comprising a recombinant C1qR binding domain. The C1qR binding domain may bind a site comprising five collagen repeats (Gly-X-Y triplets) (Malhotra, R. *et al.*, Biochem. J., 293: 15-19).

Also provided according to the present invention is the use of an inhibitor of the C1qR binding domain to inhibit CUB functionality. Such an inhibitor may of course be any molecule or other chemical agent which is capable of inhibiting the activation of the C1q receptor. Examples of such inhibitors include recombinant C1qR binding domains which competitively inhibit the binding of C1q to C1qR and thereby inhibit the activation of the C1q receptor.

The identification of the C1qR binding domain as a CUB domain provides a wide range of previously unidentified functionality for the C1q receptor and inhibitors thereof. Inhibition of the C1qR binding domain allows inhibition of the classical and novel lectin pathway of complement activtion and provides therapeutic potential in all such diseases in which complement activation is involved in the initiation and maintenance of inflammation, for example myocardial infarction, brain ischemia (stroke), gut ischemia, rheumatoid arthritis, systemic lupus erythematosus, burns and immune complex nephritis. The C1qR binding domain may also be used to inhibit the binding of β-Amyloid to C1q, thereby inhibiting the formation of amyloid plaques in Alzheimers disease. Additi nal CUB functionality includes the ability to bind carbohydrate domains of molecules, for example of collagens, and to cause opsonisation.

Thus the present invention also provides the use of an inhibitor of the C1qR binding domain according to the present invention in the manufacture of a medicament for the treatment of complement activation involved in the initiation and maintenance of inflammation, for example for the treatment of myocardial infarction, brain ischemia (stroke), gut ischemia, rheumatoid arthritis, systemic lupus erythematosus, burns, or immune complex nephritis. Also provided is the use of an inhibitor of the C1qR binding domain according to the present invention in the manufacture of a medicament for the treatment of amyloid plaques in Alzheimers disease.

Various C1qR binding domains have been identified by the present inventors, namely those of humans (Figure 1), mice (Figure 2) and rats (Figure 3). Thus the C1qR binding domain may have the sequence of any one of SEQ ID NOs: 1-3. Obviously, the sequence may be partially modified to retain CUB domain functionality yet have a sequence which is different from the one from which it was derived, i.e. one of SEQ ID NOs: 1-3, and the present invention encompasses the use of such partially modified domains. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues. Substitutions may be conserved substitutions. Hence the partially modified molecule may be a homologue of the molecules from which it was derived. It may, for example, have at least 40% homology with the molecule from which it was derived. It may for example have at least 50, 60, 70, 80, 90 or 95% homology with the molecule from which it was derived. An example of a homologue is an allelic mutant.

Also provided according to the present invention is a method of treatment of the human or animal body comprising the use of a C1qR binding domain or an inhibitr thereof according to the present invention.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, forms of C1qR binding domain. Of the figures:

Figure 1 shows the DNA sequence (SEQ ID NO: 4) and derived amino acid sequence (SEQ ID NO: 1) of human calreticulin;

Figure 2 shows the DNA sequence (SEQ ID NO: 5) and derived amino acid sequence (SEQ ID NO: 2) of mouse calreticulin;

Figure 3 shows the DNA sequence (SEQ ID NO: 6) and derived amino acid sequence (SEQ ID NO: 3) of rat calreticulin;

Figure 4 shows the amino acid sequence of CRT. Amino acid sequences were deduced from the nucleotide sequence of human CRT (McCauliffe, D.P. *et al.*, 1990, J. Clin. Invest., 85: 1379-1391.). The signal sequence residues are shown in lower case. The N- (italics), P- and C- (italics) domains are indicated. The S-domain is underlined. Domain constructs were expressed as thioredoxin fusion products;

Figure 5 shows binding of C1q to Calreticulin domains. Solid-phase bound domains, with appropriate controls, were incubated with radioiodinated C1q. Binding levels of four separate experiments, at saturation, are shown, calculated as % (bound/loaded). These percentages were then standardised against the results for C1qR;

Figure 6 shows binding of S-domain to immobilised C1q. Serial dilutions of radiolabelled S-domain were bound to immobilised C1q and BSA. After extensive washing, bound radioactivity was measured as described in Experimental section (below); and

Figure 7 shows the inhibition of S-domain-C1q interaction by collectins, C1q and C1q collagen tails. Constant levels of radiolabelled S-domain were pre-incubated with serial dilutions of unlabelled C1q, C1q tails and collectin proteins. The incubation mixture was bound to, and eluted from, solid phase C1q.

EXPERIMENTAL

Recombinant CRT domains to be tested for C1q and collectin binding function were produced from a cDNA clone (phCRT-1) isolated from a human umbilical vein endothelial cell cDNA library (Stuart, G.R. *et al.*, 1996, Exp. Lung Res.,22: 467-487; Stuart, G.R. *et al.*, 1996, FEBS Lett., 397: 245-249). These domains, as described below, are based upon structural predictions for the molecule and have previously been used to localise CRT function within the molecule. The amino-terminal N-domain contains the binding regions for PDI (Baksh, S. *et al.*, 1995, J. Biol. Chem., 270(52): 31338-31344), Zn2+ (Baksh, S. *et al.*, 1995, FEBS Lett., 376(1-2): 53-57) and integrins (Leung-Hagesteijn, C.Y. *et al.*, 1994, J. Cell Sci., 107 (Pt 3): 589-600.). The proline-rich central P-domain contains the high affinity Ca2+ binding site (Baksh, S. and Michalak, M., 1991, J. Biol. Chem., 266: 21458-21465) and the lectin site (D. Williams, cited in Krause, K.H. and Michalak, M., 1997, Cell, 88(4): 439-443) within two sets of highly conserved repeats. The acidic C-domain contains the ER-retention terminal KDEL signal (McCauliffe, D.P. *et al.*, 1990, J. Clin. Invest., 85: 1379-1391) and the low affinity Ca2+ binding site (Baksh, S. and Michalak, M., 1991, J. Biol. Chem., 266: 21458-21465). Previous studies have indicated that the C1q binding site lies across the intersection of the N and P-domains (Stuart, G.R. *et al.*, 1996, FEBS Lett., 397: 245-249). Within this region we have identified and expressed a 123 amino acid region containing a putative C1r/C1s (also termed CUB) module (Day, A.J. *et al.*, 1993, Behring Inst. Mitt., 93: 31-40) based upon amino acid sequence alignments. We termed this segment the S-domain and show here that it contains the C1q and collectin-binding site of C1qR/CRT.

Purification and Radioiodination of C1qR, C1q and Collectins

Native C1qR was purified from human U937 cells as previously described (Malhotra, R. *et al.*, 1993, Immunology, 78: 341-348). C1qR and S-domain samples were iodinated by the Iodogen method (Fraker, P.J. and Speck, J.C. Jr., 1978, Biochem. Biophys. Res. Commun., 80: 849-857). C1q was purified as previously described (Reid, K.B.M., 1981,

Methods in Enzymology, 80: 16-25) and radioiodinated as described by Bolton & Hunter (Bolton, A.E. and Hunter, W.M., 1973, Biochem. J., 133: 529-539) as this method of iodination causes less damage to large, oxidation-sensitive molecules such as C1q than the more frequently utilised Iodogen method (Stuart, G.R. *et al.*, 1996, Exp. Lung Res.,22: 467-487). Radiolabelled proteins were stored at 4 °C. C1q collagen tails were prepared as described by Reid (Reid, K.B.M., 1976, Biochem. J., 155: 5-17). Collectins were purified as previously described (Malhotra, R. *et al.*, 1990, J. Exp. Med., 172: 955-959; Holmskov, U. *et al.*, 1995, Biochem. J., 305: 889-896).

Prokaryotic expression of recombinant Calreticulin domains

Given that CRT, C1r and C1s all interact with C1q, a sequence comparison was performed to investigate the structural basis for this interaction. A region that may correspond to a CUB module was identified in CRT and was analyzed by multiple sequence alignment as described previously (Day, A.J. *et al.*, 1993, Behring Inst. Mitt., 93: 31-40). This region, termed the S region (C1s-like (CUB) domain), spans the intersection of the N and P-domains (residues 160-283). A 1.9kb cDNA clone for CRT (phCRT-1) was isolated from a human umbilical vein endothelial cell library in the eukaryotic expression vector CDM8 (Aruffo, A. and Seed, B., 1987, PNAS USA, 84: 8573-8577). Sequence analysis revealed that phCRT-1 comprised the complete coding sequence for CRT with absolute identity to the previously published human CRT sequence (McCauliffe, D.P. *et al.*, 1990, J. Clin. Invest., 85: 1379-1391).

The Thiobond expression system was used to produce N, P, C and S-domains of C1qR/CRT (representing the N-terminal region, the proline-rich central region, the C-terminal region, and a region spanning the intersection of the N and P-domains (as described above) (Figure 4). The individual domains were expressed as thioredoxin fusion proteins in E. Coli using the plasmid pTrxfus (Invitrogen BV, Leek, Netherlands) as described previously (Stuart, G.R. *et al.*, 1996, FEBS Lett., 397: 245-249).

Samples were assayed for recombinant calreticulin domain expression by SDS-PAGE (Laemmli, U.K., 1970, Nature,227: 680-685) and by Western blotting with rabbit antisera to:(1) whole C1qR (raised against human C1qR purified from U937 cells (Malhotra, R. *et al.*, 1993, Immunology, 78: 341-348); (2) CRT C-terminal region (raised against a GST fusion protein containing the final 18 residues of recombinant human CRT), and (3) CRT N-terminal region (raised against a GST fusion protein containing residues 7-18 of recombinant human CRT).

Interaction of immobilised recombinant human C1qR/CRT domains with radiolabelled C1q

Binding experiments with the C1qR/CRT domains were performed throughout in low salt (10mM potassium phosphate, 0.5mM EDTA (pH 7.4)) in order to maximise the ionic interaction with C1q.

Microtitre plates were coated with the N-, P-, C- and S-domains and with three controls, C1qR, BSA and Thioredoxin, (8mg/ml in 35mM NaHCO3, 15mM Na2CO3 pH 9.6) for 2hr at 37 °C. Non-specific interactions were blocked by incubation with 10mM potassium phosphate, 0.5mM EDTA pH 7.4 containing BSA (10mg/ml). Any free -SH groups in the samples, due to the presence of the thioredoxin fusion protein, were blocked by a brief washing step using the phosphate buffer containing 2mM iodoacetamide. After washing, serial dilutions of radioiodinated C1q (in 10mM potassium phosphate, 0.5mM EDTA, pH 7.4) were added to the wells and incubated for 2h at 37 °C. Wells were washed three times with phosphate buffer and bound radioactivity eluted with 100ml 4M NaOH and measured.

Concentration dependent binding of radiolabelled S-domain to immobilised C1q

C1q binds to the Fc regions of IgG.

This property was utilised in order to correctly orient the C1q on microtitre plates. Breakable microtitre plates (Life Sciences International) were coated with rabbit Fc (5mg

per well in 35mM $NaHCO_3$, 15mM Na_2CO_3 pH 9.6) . Non-specific sites were blocked as described above, and the wells were incubated with C1q (5 mg per well in 10mM potassium phosphate buffer). Certain wells were also coated with BSA as a negative control. After further washing, serial dilutions of radioiodinated S-domain (in 10mM potassium phosphate, 0.5mM EDTA, pH 7.4) were added to the wells and incubated for 2h at 37 °C. Wells were washed three times with the phosphate buffer and bound radioactivity in the individual wells measured.

Competitive inhibition of the S-domain-C1q interaction by fluid phase C1q, collectins and C1q collagen tails

C1q was immobilised onto Fc-coated microtitre plates as described above. Non-specific binding was blocked by incubation with 10mM potassium phosphate, 0.5mM EDTA (pH 7.4) containing BSA (10mg/ml). Serial dilutions of the collectins (SP-A, MBL, SP-D, CL43), C1q, C1q tails and BSA (maximum quantity = 9mg/well) were prepared in 10mM potassium phosphate, 0.5mM EDTA (pH 7.4). Each dilution (100ml) was then incubated for 1h at 37 °C with a constant level of radiolabelled S-domain and loaded onto the plate.Following 2 hours incubation at 37 °C, wells were extensively washed and bound radioactivity measured.

Results

Amino acid sequence alignments

A region spanning the intersection of the N- and P-domains of CRT has previously been implicated in C1q binding (Stuart, G.R. *et al.*, 1996, FEBS Lett., 397: 245-249). Amino acid sequence alignment of a region within CRT (residues 160-283) showed little primary homology to a CUB module, but the region is in fact a CUB moodule. Two CUB modules, together with an EGF module, form a binding region within C1r2C1s2 for the collagenous tails of C1q. C1qR competes with C1r2C1s2 for binding to C1q, implying a similarity in C1q binding sites on C1r, C1s and C1qR (Sobel, A.T. and Bokisch, V.A., in: Membrane receptors of lymphocytes (M. Seligman, FL Preud'homme, FM Kourilsky

eds). North Holland Publishing Co., Amsterdam, p151, 1975; Van den Berg, R.H. *et al.*, 1995, Eur. J. Immunol., 25(8): 2206-2210). This segment, the S-domain (residues 160-283, see Figure 4), was tested for C1q binding.

Interaction of recombinant human Calreticulin domains with C1q

N-, P-, C-, and S-domains of human CRT were expressed as thioredoxin fusion proteins. Correct expression was verified by SDS-PAGE and Western blotting. Figure 5 summarises the results of four separate solid phase direct binding experiments. Significant binding to radioiodinated C1q was observed for C1qR, the S-domain, the P-domain, and, to a lesser extent, the N-domain. The C-domain showed no binding.

Serial dilutions of radioiodinated S-domain were incubated with immobilised C1q and BSA (Figure 6). Concentration-dependent, saturable binding was observed to C1q but not to BSA.

Competitive inhibition of C1q-S-domain interaction by C1q tails and Collectins.

C1q was immobilised on microtitre plates by interaction with solid phase Fc. Figure 7 shows the results of competitive inhibition of the S-domain-C1q interaction. As expected, native fluid-phase C1q demonstrates concentration-dependent inhibition. C1q tails also cause inhibition, indicating that the interaction of the S-domain with C1q is via the collagenous C1q tails. Inhibition studies with the collectin proteins demonstrated that SP-A, MBL and CL43 interact with the S-domain, via the same, or an overlapping binding site as C1q. SP-D and BSA did not inhibit the S-domain-C1q interaction.

CLAIMS

1. The use of a C1qR binding domain as a CUB domain.

2. The use of a C1qR binding domain according to claim 1, the binding domain forming part of a C1q receptor.

3. The use of a C1qR binding doamin according to either one of claims 1 or 2, the C1qR binding domain being recombinant.

4. The use of an inhibitor of the C1qR binding domain to inhibit CUB functionality.

5. The use of an inhibitor of the C1qR binding domain according to claim 4, the inhibitor comprising a C1qR binding domain.

6. The use of an inhibitor of the C1qR binding domain according to either one of claims 4 or 5 in the manufacture of a medicament for the treatment of complement activation involved in the initiation and maintenance of inflammation.

7. The use of an inhibitor of the C1qR binding domain according to claim 6 in the manufacture of a medicament for the treatment of myocardial infarction, brain ischemia (stroke), gut ischemia, rheumatoid arthritis, systemic lupus erythematosus, burns, or immune complex nephritis.

8. The use of an inhibitor of the C1qR binding domain according to either one of claims 4 or 5 in the manufacture of a medicament for the treatment of amyloid plaques in Alzheimers disease.

9. The use of a C1qR binding domain or inhibitor thereof accoring to any one of the preceding claims, the C1qR binding domain having the sequence of any one of SEQ ID NOs: 1-3 or a partially modified form thereof.

10. A method of treatment of the human or animal body comprising the use of a C1qR binding domain or an inhibitor thereof according to any one of the preceding claims.

ABSTRACT

The present invention concerns novel uses of the C1q Receptor (C1qR) binding domain and inhibitors thereof.

[redacted]DNA Strider 1.0 ### Wednesday, June 11, 1997 3:58:49 pm

human [redacted]alreticulin CUB domain -> 1-phase Translation

DNA sequence 366 b.p. CGTTGCAAGGAT ... ATCGACAACCCA linear

```
1   /   1                                31   /   11
CGT TGC AAG GAT GAT GAG TTT ACA CAC CTG TAC ACA CTG ATT GTG CGG CCA GAC AAC ACC
arg cys lys asp asp glu phe thr his leu tyr thr leu ile val arg pro asp asn thr
61   /  21                               91   /   31
TAT GAG GTG AAG ATT GAC AAC AGC CAG GTG GAG TCC GGC TCC TTG GAA GAC GAT TGG GAC
tyr glu val lys ile asp asn ser gln val glu ser gly ser leu glu asp asp trp asp
121  /  41                               151  /   51
TTC CTG CCA CCC AAG AAG ATA AAG GAT CCT GAT GCT TCA AAA CCG GAA GAC TGG GAT GAG
phe leu pro pro lys lys ile lys asp pro asp ala ser lys pro glu asp trp asp glu
181  /  61                               211  /   71
CGG GCC AAG ATC GAT GAT CCC ACA GAC TCC AAG CCT GAG GAC TGG GAC AAG CCC GAG CAT
arg ala lys ile asp asp pro thr asp ser lys pro glu asp trp asp lys pro glu his
241  /  81                               271  /   91
ATC CCT GAC CCT GAT GCT AAG AAG CCC GAG GAC TGG GAT GAA GAG ATG GAC GGA GAG TGG
ile pro asp pro asp ala lys lys pro glu asp trp asp glu glu met asp gly glu trp
301  / 101                              331  /  111
GAA CCC CCA GTG ATT CAG AAC CCT GAG TAC AAG GGT GAG TGG AAG CCC CGG CAG ATC GAC
glu pro pro val ile gln asn pro glu tyr lys gly glu trp lys pro arg gln ile asp
361  / 121
AAC CCA
asn pro
```

Fig. 1

DNA Strider 1.0 ## Wednesday, June 11, 1997 4:19:41 pm

mouse calreticulin CUB domain -> 1-phase Translation

DNA sequence 324 b.p. CGGTGTAAGGAT ... ATTCAAAATCCT linear

```
1   /   1                                31   /   11
CGG TGT AAG GAT GAT GAA TTC ACA CAC CTA TAC ACA CTG ATT GTG CGG CAA GAC AAC ACC
arg cys lys asp asp glu phe thr his leu tyr thr leu ile val arg gln asp asn thr
61   /   21                              91   /   31
TAT GAG GTG AAA ATT GAC AAC AGC CAG GTG GAG TCA GGC TCC TTG GAG GAT GAT GGG GAC
tyr glu val lys ile asp asn ser gln val glu ser gly ser leu glu asp asp gly asp
121  /   41                             151  /   51
TTT CTG CCA CCC AAG AAG ATA AAG GAC CCT GAT GCT GCC AAG CCG GAA GAC TGG GAT GAA
phe leu pro pro lys lys ile lys asp pro asp ala ala lys pro glu asp trp asp glu
181  /   61                             211  /   71
CGA GCC AAG ATC GAT GAC CCC ACA GAT TCC AAG CCT GAG GAC TGG GAC AAG CCA GAG CAC
arg ala lys ile asp asp pro thr asp ser lys pro glu asp trp asp lys pro glu his
241  /   81                             271  /   91
ATC CCT GAC CCT GAT GCT AAG AAG CCT GAG GAC TGG GAT GAA GAG ATG GAT GGA GAG TGG
ile pro asp pro asp ala lys lys pro glu asp trp asp glu glu met asp gly glu trp
301  /  101
GAA CCA CCA GTG ATT CAA AAT CCT
glu pro pro val ile gln asn pro
```

Fig. 2

DNA Strider 1.0 ### Wednesday, June 11, 1997 4:33:06 pm

ra[redacted]calreticulin domain -> 1-phase Translation

DNA sequence 324 b.p. CGGTGTAAGGAT ... ATTCAAAATCCT linear

```
1   /   1                                   31   /   11
CGG TGT AAG GAT GAT GAA TTC ACA CAT CTA TAC ACG CTG ATT GTG CGG CCA GAC AAC ACC
arg cys lys asp asp glu phe thr his leu tyr thr leu ile val arg pro asp asn thr
61   /   21                                 91   /   31
TAC GAG GTG AAA ATT GAC AAC AGC CAG GTG GAG TCG GGC TCC TTG GAG GAT GAT TGG GAC
tyr glu val lys ile asp asn ser gln val glu ser gly ser leu glu asp asp trp asp
121  /   41                                 151  /   51
TTT CTG CCG CCC AAG AAG ATT AAG GAT CCT GAC GCT GCC AAG CCA GAA GAC TGG GAT GAA
phe leu pro pro lys lys ile lys asp pro asp ala ala lys pro glu asp trp asp glu
181  /   61                                 211  /   71
CGA GCC AAG ATT GAT GAC CCC ACA GAT TCC AAG CCT GAG GAC TGG GAC AAG CCA GAG CAC
arg ala lys ile asp asp pro thr asp ser lys pro glu asp trp asp lys pro glu his
241  /   81                                 271  /   91
ATC CCT GAC CCT GAT GCT AAG AAG CCT GAG GAC TGG GAC GAA GAG ATG GAT GGA GAG TGG
ile pro asp pro asp ala lys lys pro glu asp trp asp glu glu met asp gly glu trp
301  /  101
GAA CCA CCA GTG ATT CAA AAT CCT
glu pro pro val ile gln asn pro
```

Fig. 3

```
  1 m l l s v p l l l g l l g l a v a E P A V Y F K E Q F L D G D G
 33 W T S R W I E S K H K S D F G K F V L S S G K F Y G D E E K D K
 65 G L Q T S Q D A R F Y A L S A S F E P F S N K G Q T L V V Q F T
 97 V K H E Q N I D C G G G Y V K L F P N S L D Q T D M H G D S E Y
129 N I M F G P D I C G P G T K K V H V I F N Y K G K N V L I N K D
161 I R C K D D E E T H L Y T L I V R P D N T Y E V K I D N S Q V E
193 S G S L E D D W D F L P P K K I K D P D A S K P E D W D E R A K
225 I D D P T D S K P E D W D K P E H I P D P D A K K P E D W D E E
257 M D G E W E P P V I Q N P E Y K G E W K P R Q I D N P D Y K G T
289 W I H P E I D N P E Y S P D P S I Y A Y D N F G V L G L D L W Q
321 V K S G T I F D N F L I T N D E A Y A E E F G N E T W G V T K A
353 A E K Q M K D K Q D E E Q R L K E E E E D K K R K E E E E A E D
385 K E D D E D K D E D E E D E E D K E E D E E E D V P G Q A K D E L
```

Fig. 4

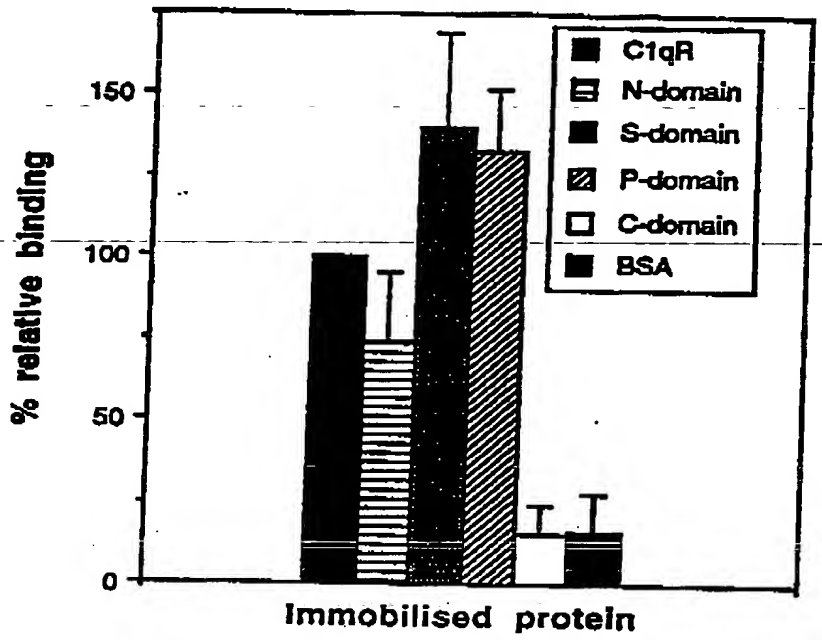
C1qR
N-domain
S-domain
P-domain
C-domain
BSA
150
100
50
0
% relative binding
Immobilised protein

Fig. 5

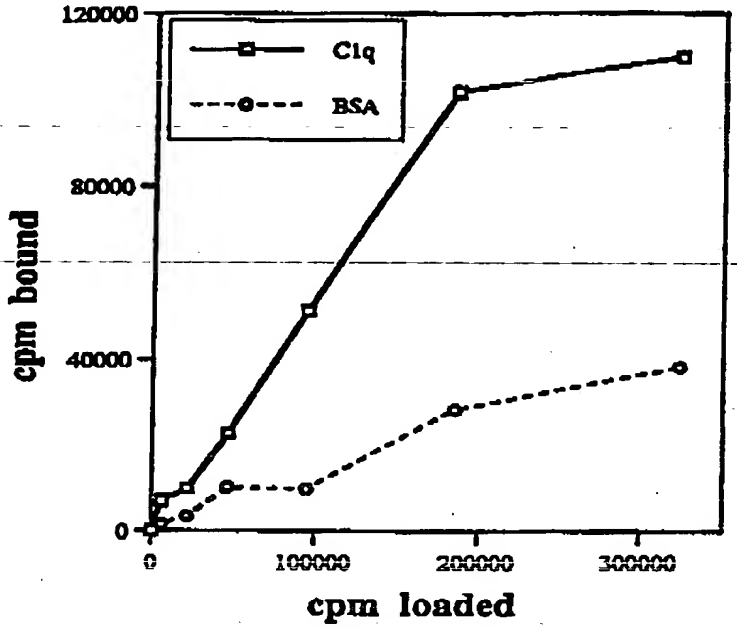
120000
80000
40000
0
cpm bound
Clq
BSA
0
100000
200000
300000
cpm loaded

Fig. 6

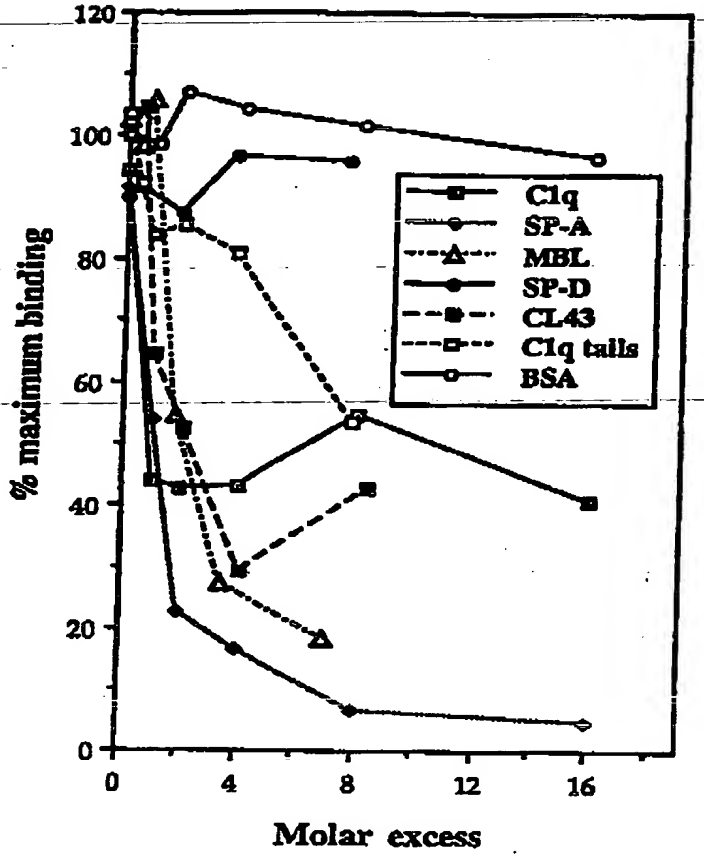
120
100
80
60
40
20
0
% maximum binding
0
4
8
12
16
Molar excess
C1q
SP-A
MBL
SP-D
CL43
C1q tails
BSA

Fig. 7